5750338

FIG. la

CELLS
WITHIN   TARGET DNA
CLINICAL
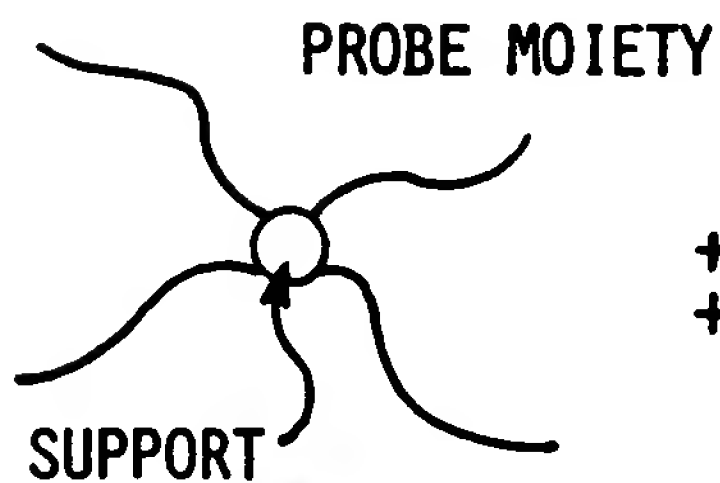SAMPLE

SOLUBLIZING AGENTS
& DENATURATION
OF NUCLEIC ACID
STEP 1

SAMPLE NUCLEIC ACID & CELLULAR DEBRI & IMPURITIES

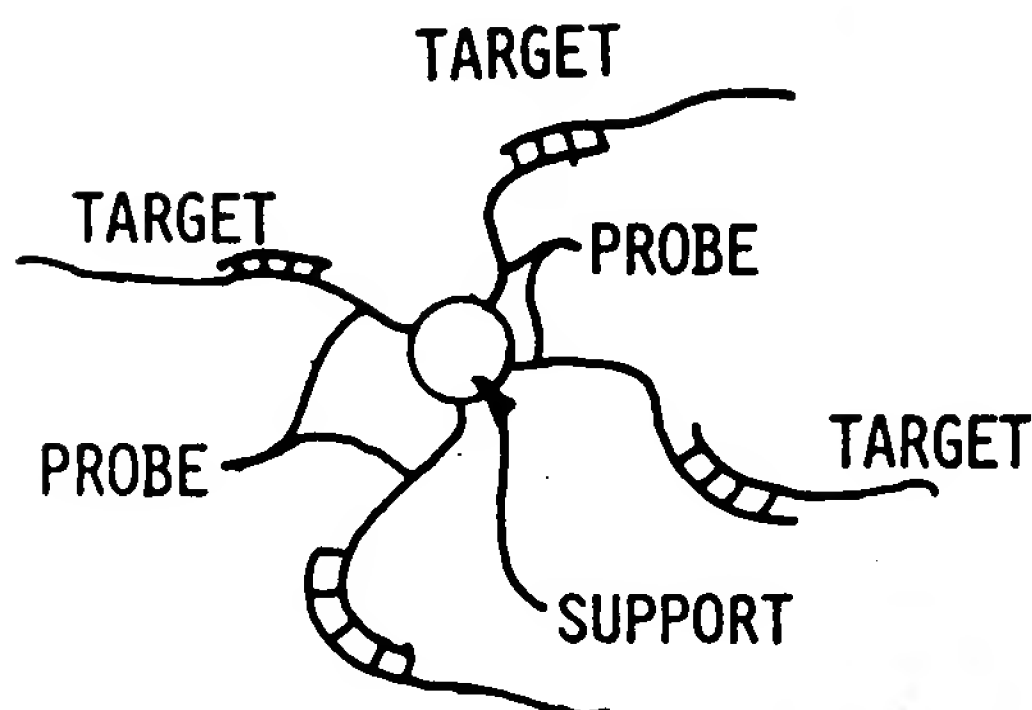STEP 2   RETRIEVABLE SUPPORT
              WITH PROBE MOIETY

              PROBE MOIETY

SAMPLE
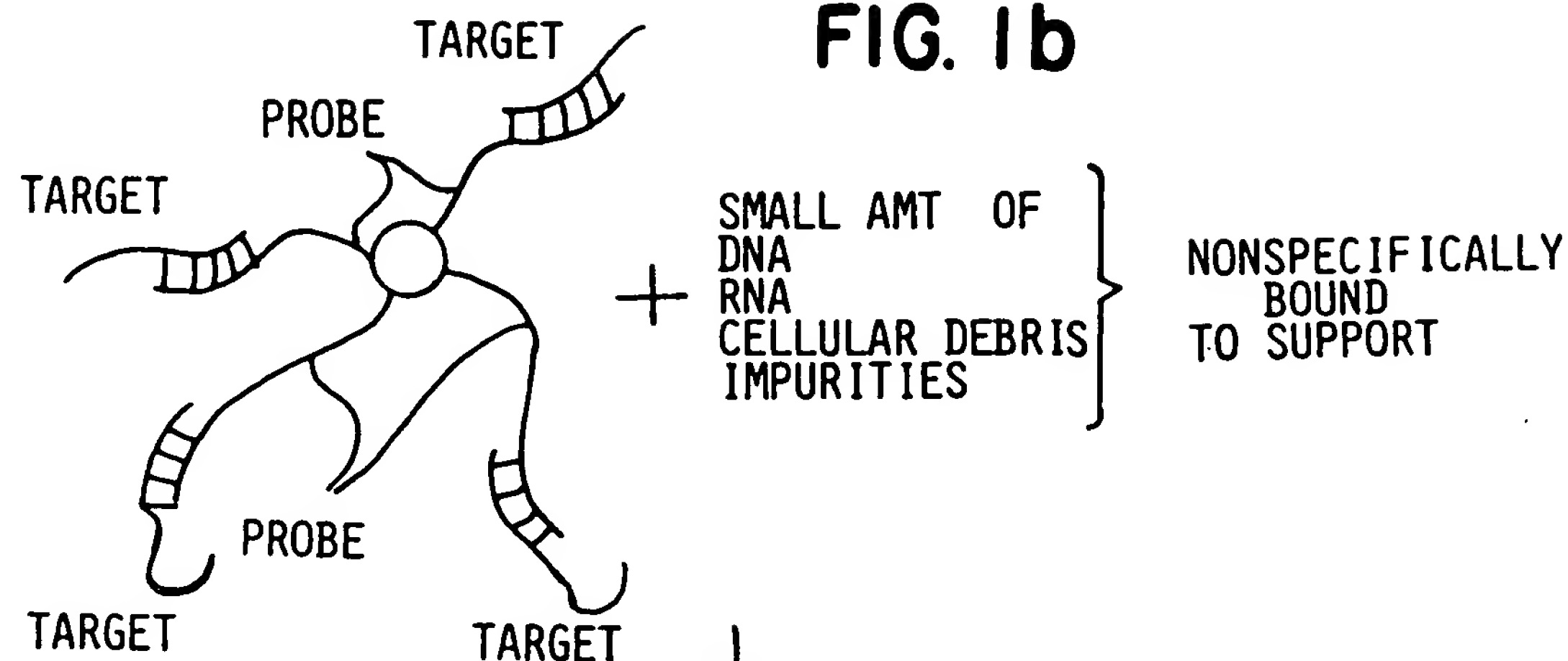NUCLEIC ACID +   + CELLULAR DEBRIS
                                   + IMPURITIES

STEP 3
        SUPPORT

              BINDING CONDITIONS

        TARGET
TARGET   PROBE
                                   LARGE AMOUNT OF
                                   DNA
                          +   RNA
PROBE   TARGET   CELLULAR DEBRIS
                                   IMPURITIES
        SUPPORT

STEP 4

FIG. 1b

TARGET

PROBE

TARGET

$+$ SMALL AMT OF
DNA
RNA
CELLULAR DEBRIS
IMPURITIES

NONSPECIFICALLY
BOUND
TO SUPPORT

PROBE

TARGET   TARGET

<u>STEP 5</u>

DENATURATION

TARGET

TARGET

PROBE

SUPPORT

$+$ DNA
RNA
CELLULAR
      DEBRIS
IMPURITIES

NONSPECIFICALLY
BOUND
TO SUPPORT
OR NOW IN
SOLUTION
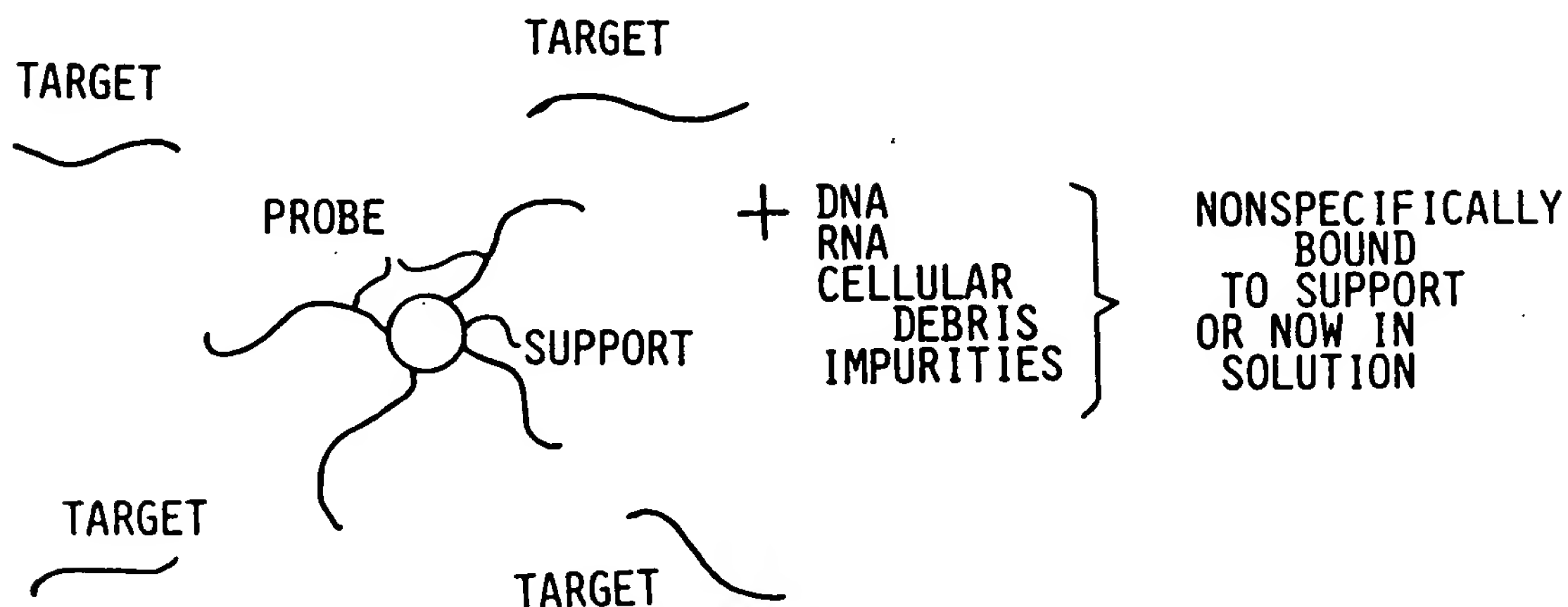
TARGET
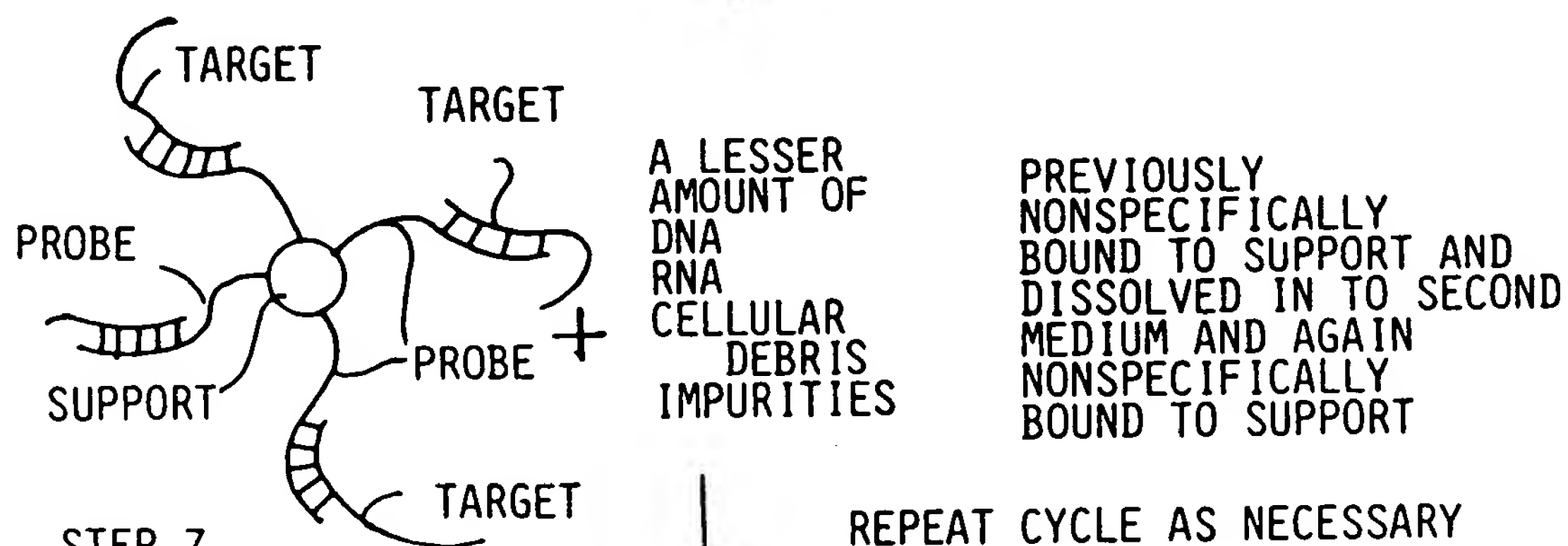
TARGET

<u>STEP 6</u>

SEPARATION OF OLD SUPPORT
FROM SECOND MEDIUM AND
INTRODUCTION OF NEW SUPPORT
UNDER BINDING CONDITIONS

TARGET

TARGET

PROBE

PROBE $+$

SUPPORT

TARGET

<u>STEP 7</u>

A LESSER
AMOUNT OF
DNA
RNA
CELLULAR
      DEBRIS
IMPURITIES

PREVIOUSLY
NONSPECIFICALLY
BOUND TO SUPPORT AND
DISSOLVED IN TO SECOND
MEDIUM AND AGAIN
NONSPECIFICALLY
BOUND TO SUPPORT

REPEAT CYCLE AS NECESSARY

<u>STEP 8</u>

DETECT TARGET

FIG. 2a

CLINICAL SAMPLE

SOLUBILIZING AGENTS
AND REAGENT·

STEP 1

LABEL MOIETIES

P_1 L_1 + P_2 L_2 +
SOLUBILIZING AGENTS
IMPURITIES
CELLULAR MATERIAL

TARGET +

REAGENT

BINDING CONDITIONS

STEP 2

TARGET

$3'$ P_1 P_2 $5'$ T_2
L_1 $3'$ $3'$ + P_1 L_1 P_2 L_2 +
SOLUBILIZING AGENTS
IMPURITIES
CELLULAR MATERIAL

RETRIEVABLE SUPPORT WITH
A_1 MOIETY UNDER BINDING CONDITIONS

STEP 3

TARGET

L_2 P_2 A_1 S_1
P_1 L_1 $\overline{A}_1$ A_1
L_1 P_1
+ P_1 L_1 P_2 L_2 +
SOLUBILIZING AGENTS
IMPURITIES
CELLULAR MATERIAL

IMMOBILIZE RETRIEVABLE SUPPORT
WASH IMMOBILIZED SUPPORT
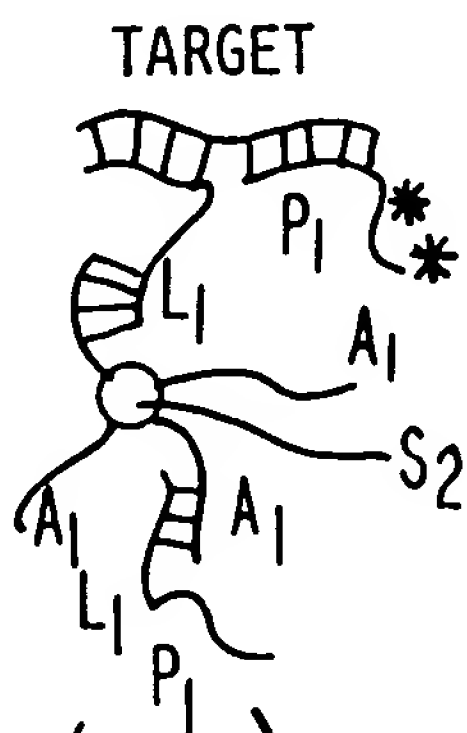
STEP 4

FIG. 2b

TARGET

SEPARATE RETRIEVABLE
SUPPORT FROM
TARGET-PROBE
COMPLEX BY
DENATURATION

MONITOR
SAMPLE
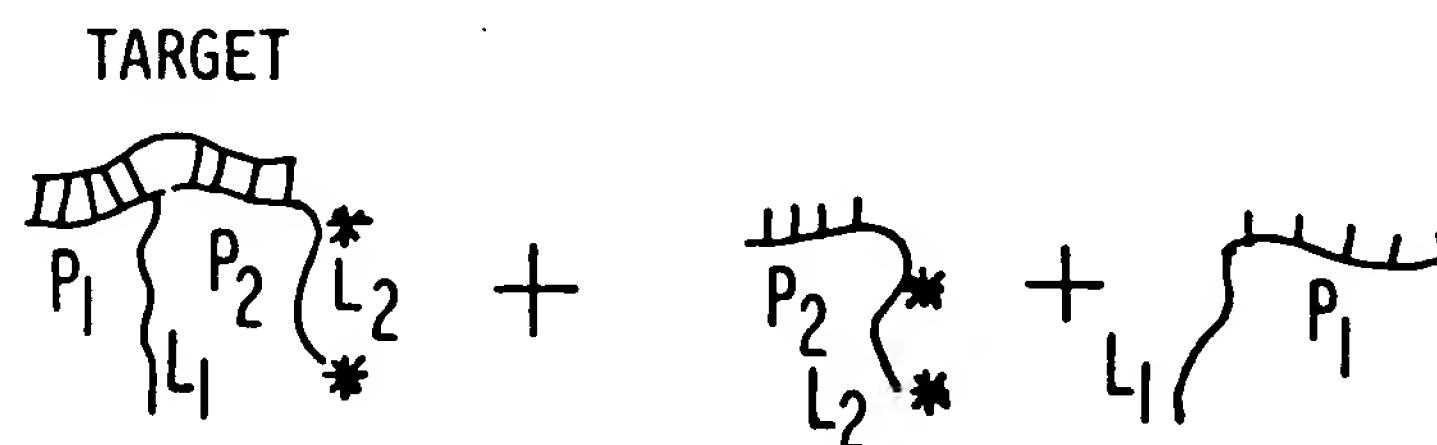FOR LABEL

STEP 5

TARGET

REMOVE
RETRIEVABLE SUPPORT

STEP 6

TARGET

TARGET

STEP 7

DETECT
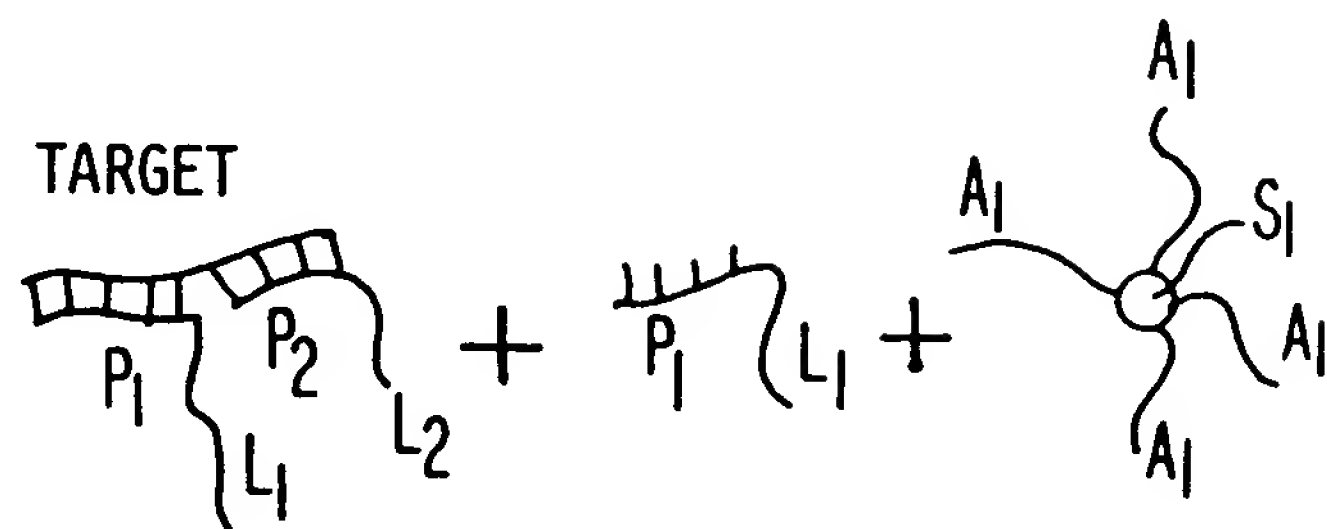
REPEAT
CYCLE

MONITOR SAMPLE
FOR LABEL

STEP 8

FIG. 3

TARGET

P_1 P_2 L_2
L_1 *   + P_2 *   + L_1 P_1
                  L_2 *
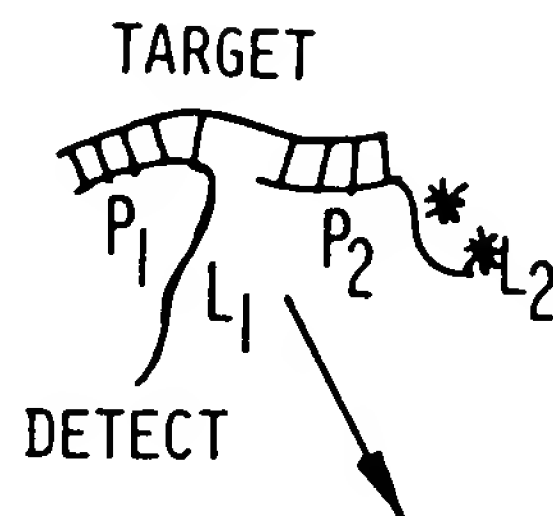
STEP 1 →

SUPPORT SELECTIVELY BINDING
TO SECOND LIGAND OF P_2
UNDER BINDING CONDITIONS

TARGET

P_1 + L_1 P_1 P_2 L_2 *   + L_2 P_2
                                              A_2
                                        L_2 * A_2 A_2 L_2 *   B
                                            P_2 P_2
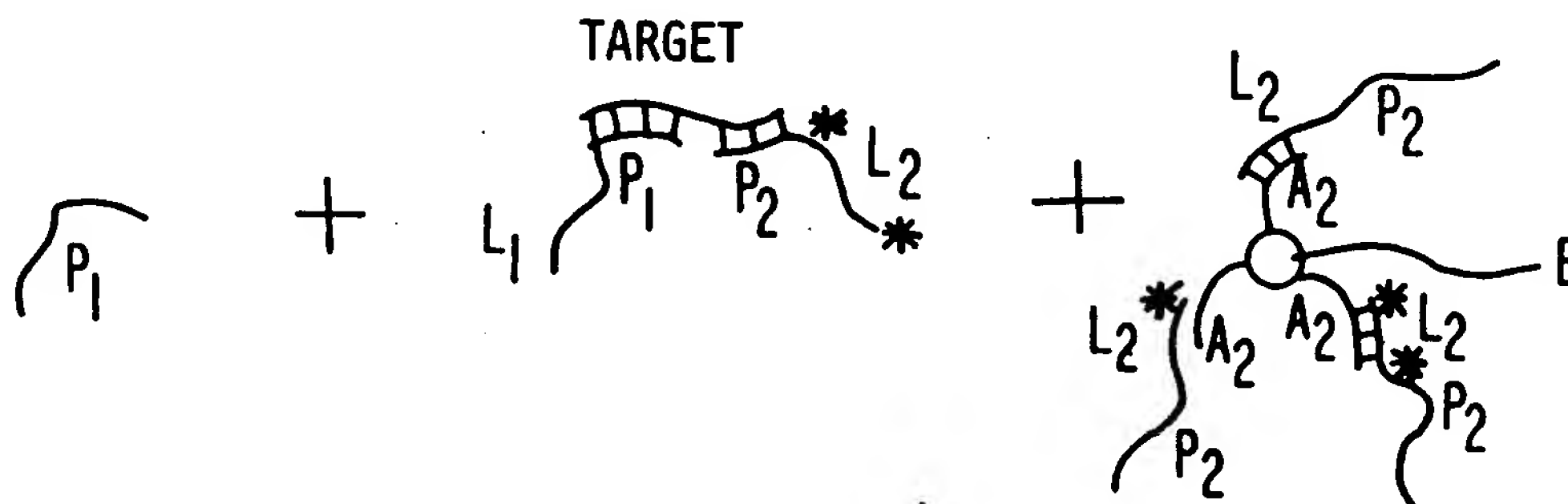
STEP 2

REMOVE RETRIEVABLE
SUPPORT (B_1)
↓

TARGET

P_1 P_2 L_2 *
L_1

MONITOR
SAMPLE
FOR LABEL   FURTHER
                   PROCESSING

STEP 3

FIG.4

TARGET DNA IN ROUGH SAMPLE

CAPTURE PROBE (REC^A PROTEIN COATED)
CAPTURE BEAD
BINDING CONDITIONS

STEP I

A
BEAD
C'   B'   A'   COMPLEMENTARY
HOMOPOLYMER TAILS

STEP 2   ISOLATE BEADS

TARGET DNA (SUBSTANTIALLY FREE OF SAMPLE
IMPURITIES, DEBRI, EXTRANEOUS
POLYNUCLEOTIDES

STEP 3   CORE RNA POLYMERASE
LOW SALT BUFFER

C'   B'   A'   TARGET DNA

$\left(\begin{array}{ccc} & & \\ C & B & A \end{array} \right)_N$ RNA COMPLEMENTARY TO TARGET DNA

STEP 4   LABEL PROBE
CAPTURE PROBE
CAPTURE SUPPORT

CAPTURE
PROBE

LABEL
PROBE
LABEL
PROBE   *
COMPLEMENTARY
HOMOPOLYMER TAILS

C   B   A

AMPLIFICATION
PRODUCT   SUPPORT

FIG. 5

TARGET DNA IN ROUGH SAMPLE

CAPTURE PROBE
CAPTURE BEAD

STEP I
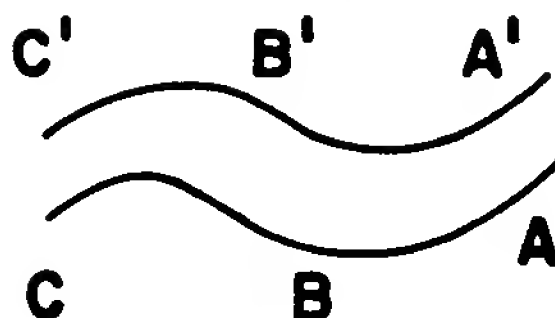
BEAD

A

C'   B'   A'

COMPLEMENTARY
HOMOPOLYMER TAILS

STEP 2   ISOLATE BEADS

TARGET DNA (SUBSTANTIALLY FREE OF
SAMPLE IMPURITIES, DEBRI,
EXTRANEOUS POLYNUCLEOTIDES)

DNA POLYMERASE
HEXAMER PRIMERS
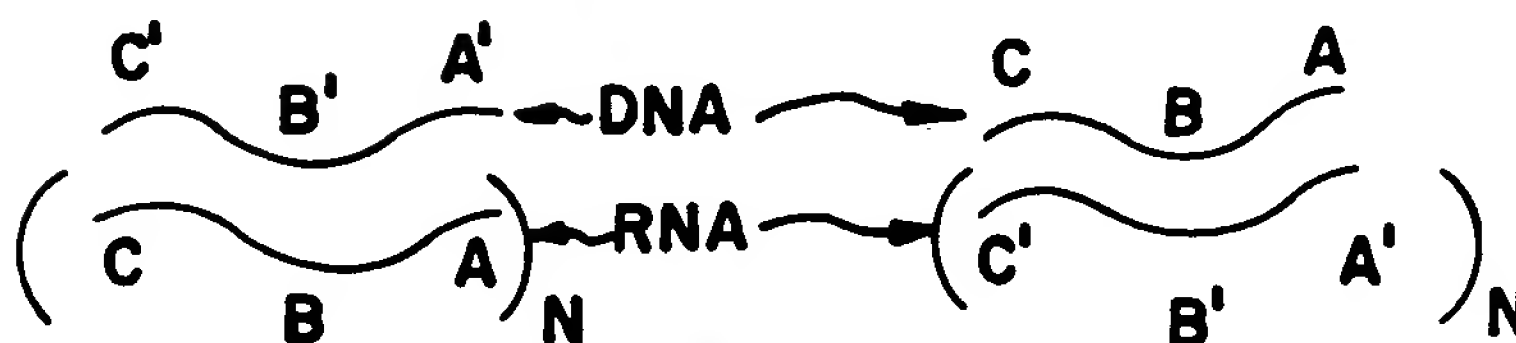
STEP 3a

C'   B'   A'

C   B   A

CORE RNA POLYMERASE
LOW SALT BUFFER

STEP 3b

C'   B'   A' — DNA →   C   B   A

(C   B   A) — RNA →   (C'   B'   A')
        N   N

STEP 4

LABELLED PROBE
CAPTURE PROBE
SUPPORT

AMPLIFICATION TARGET

C   B   A   CAPTURE
                    PROBE

SUPPORT

*C'   A

LABEL
PROBE

C'   A'

*C   B'   A   CAPTURE
                                   PROBE

TARGET/AMPLIFICATION
PRODUCT

FIG. 6

TARGET DNA IN ROUGH SAMPLE

STEP 1
                    ↓ CAPTURE PROBE
                      CAPTURE BEAD

CAPTURE
PROBE   A   ○————BEAD
TARGET   COMPLEMENTARY
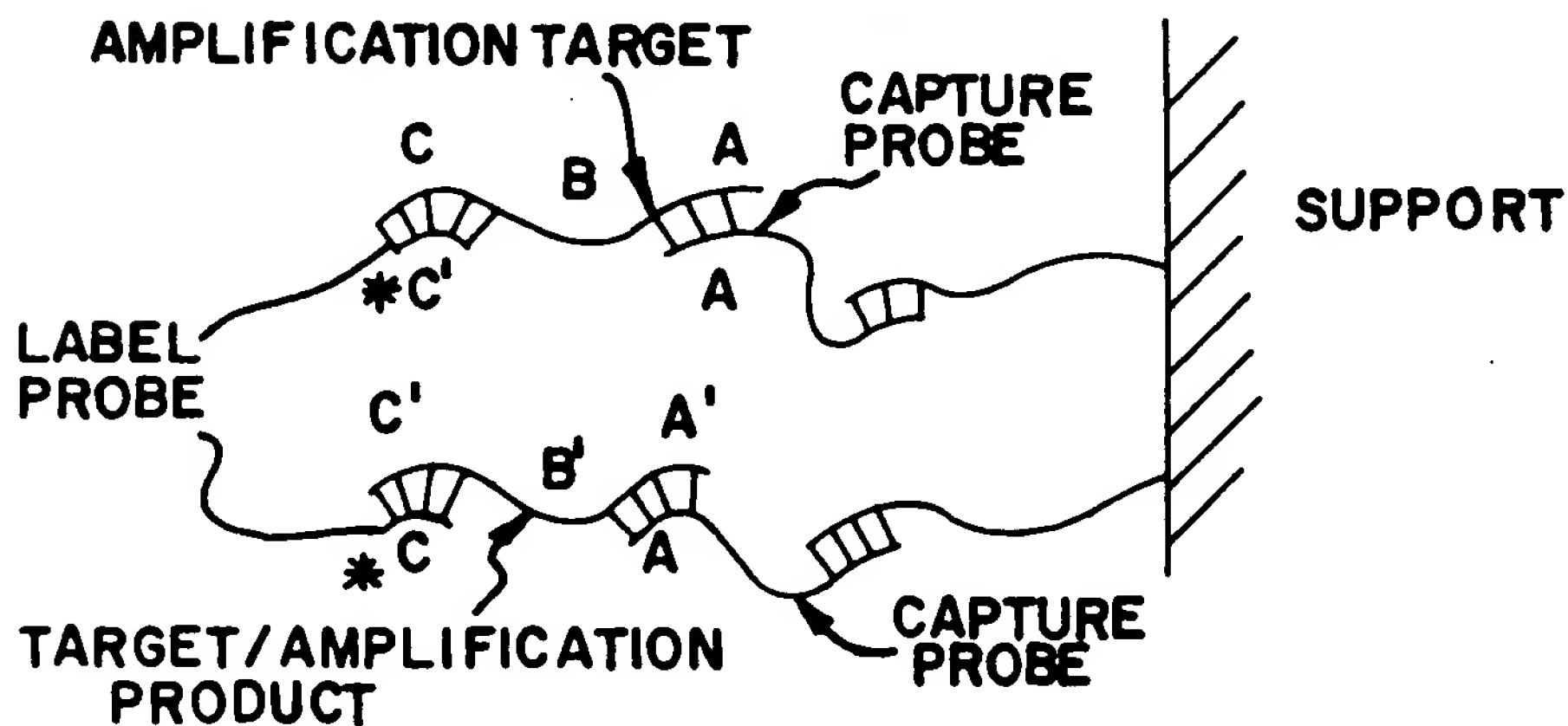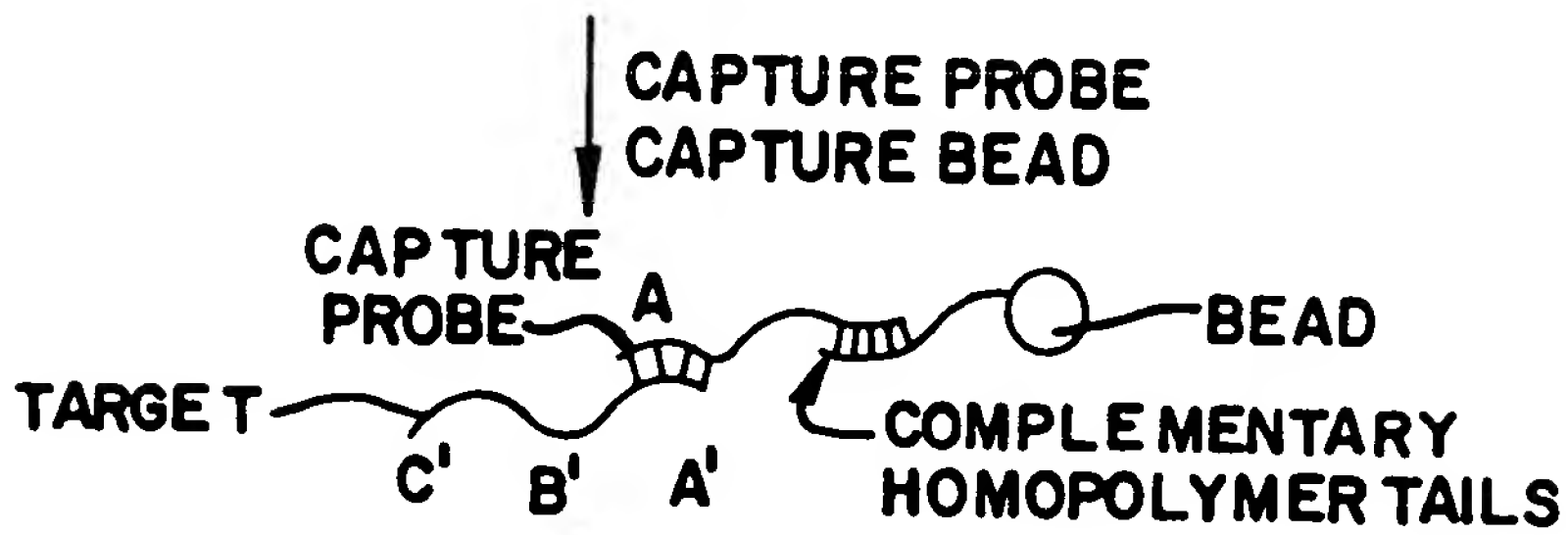   C'   B'   A'   HOMOPOLYMER TAILS

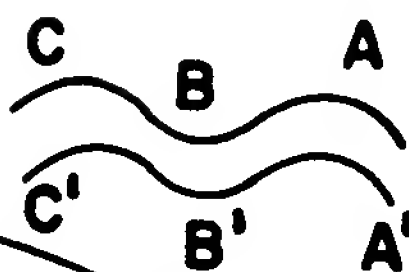STEP 2   ↓ ISOLATE BEAD

TARGET DNA   (SUBSTANTIALLY FREE OF SAMPLE
              IMPURITIES, DEBRI, AND EXTRANEOUS
              POLYNUCLEOTIDES)

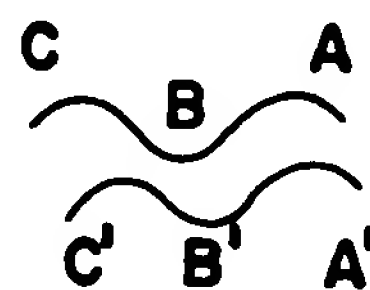STEP 3a   ↓ DNA POLYMERASE
                  HEXAMER PRIMERS

      C   B   A
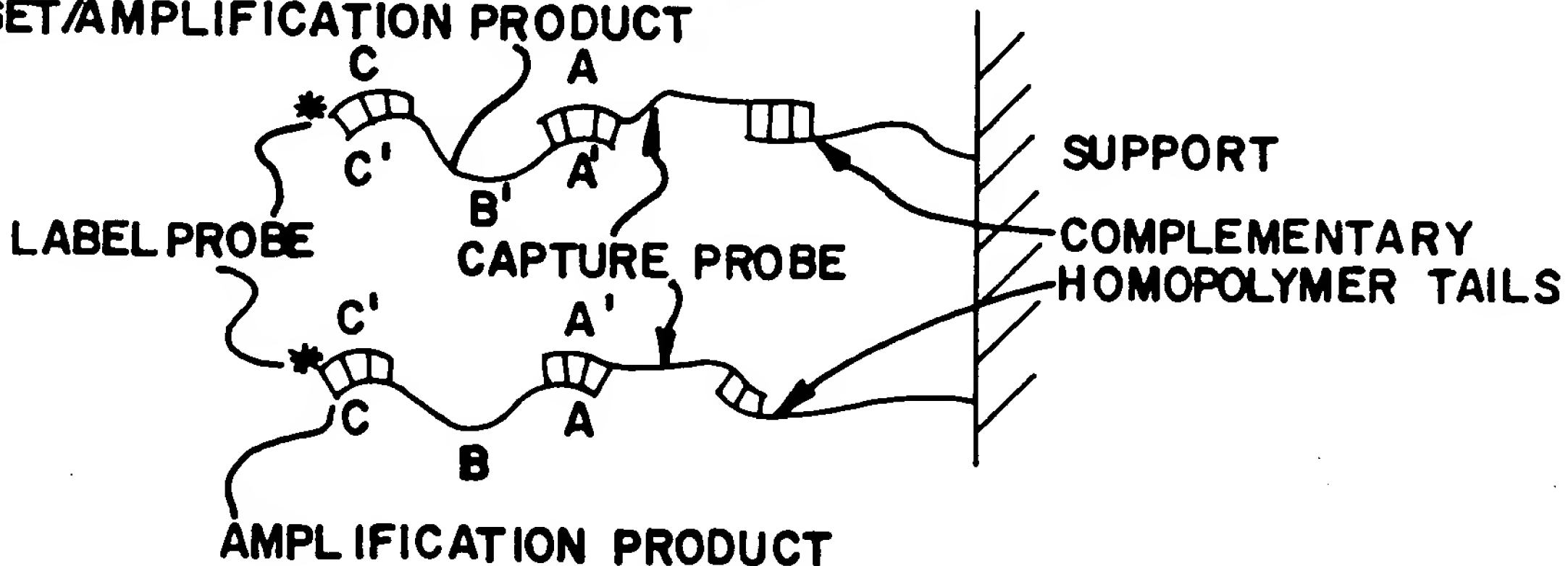   FIRST COMPLEMENT
      C'   B'   A'   TARGET

STEP 3b   ↓ 1. DENATURE
                  2. DNA POLYMERASE

SECOND COMPLEMENT——   C   B   A   C   B   A   FIRST COMPLEMENT
TARGET——   B   COMPLEMENT TO FIRST
              C' B' A'   C' B' A'
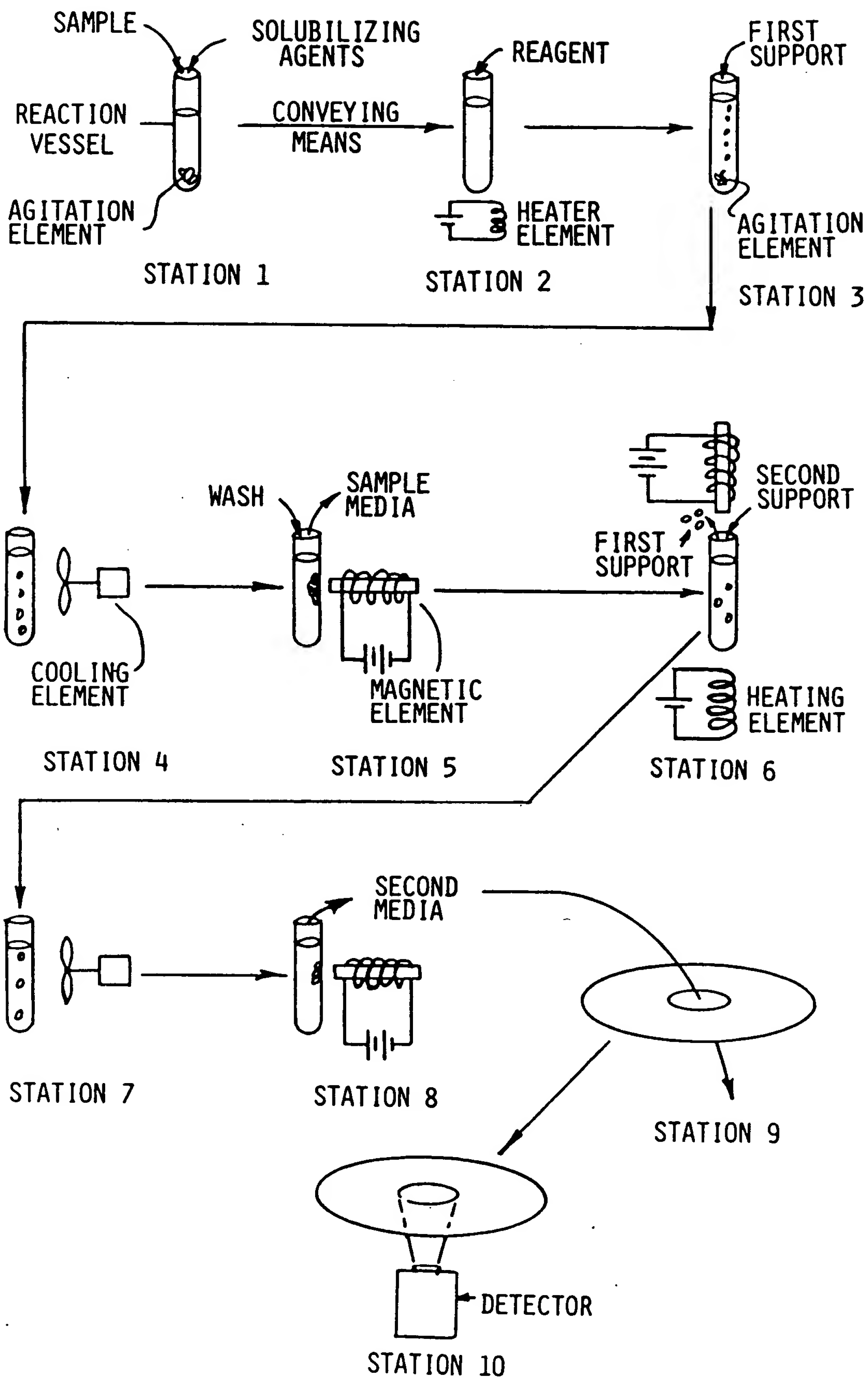
                ↓ DENATURE
                  LABEL PROBE
                  CAPTURE SUPPORT
                  CAPTURE PROBE
STEP 4

TARGET/AMPLIFICATION PRODUCT
              C   A
              C'   A'   SUPPORT
LABEL PROBE   B'
                   CAPTURE PROBE   COMPLEMENTARY
              C'   A'   HOMOPOLYMER TAILS
              C   A
                  B

AMPLIFICATION PRODUCT

FIG. 7

SAMPLE — SOLUBILIZING AGENTS — REAGENT

REACTION VESSEL — CONVEYING MEANS

AGITATION ELEMENT — HEATER ELEMENT

FIRST SUPPORT — AGITATION ELEMENT

STATION 1 — STATION 2 — STATION 3

WASH — SAMPLE MEDIA — SECOND SUPPORT — FIRST SUPPORT

COOLING ELEMENT — MAGNETIC ELEMENT — HEATING ELEMENT

STATION 4 — STATION 5 — STATION 6

SECOND MEDIA

STATION 7 — STATION 8 — STATION 9

DETECTOR

STATION 10

ELT B GENE   ELT A GENE

8Kb

PBR322- LIKE SEQUENCES

FIG. 8